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TECHNICAL NOTES

Cholesterol Esters of Skim Milk

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ABSTRACT

Cholesterol esters in raw skim milk differed in fatty acid composition from their composition in whole milk. The esters, isolated by chloroform-methanol extraction and preparative thin-layer chromatography, are characterized by an octadecadienoic acid content greater than 70 wt %. In all, unsaturated fatty acids contribute 90 wt % of the fatty acids in the cholesterol ester fraction of skim milk. These results with previous observations suggests that unsaturated lipids may be associated preferentially with the aqueous phase of milk.

INTRODUCTION

We previously determined (3) that fresh skim milk contains on a percent composition basis a disproportionate amount of unesterified octadecadienoic fatty acids relative to their content in whole milk. We undertook to determine whether this phenomenon was unique to the unesterified fatty acids or representative of other types of lipids in milk. The results on the fatty acid composition of the cholesterol esters of skim milk are reported herein and are compared with those in whole milk.

MATERIALS AND METHODS

Materials

All solvents were analytical grade, redistilled, and stored in glass. Glass wool was treated according to the procedure of Schwartz (6) and further washed with methylene chloride prior to use. Anhydrous granular sodium sulfate was washed with chloroform or methylene chloride, dried in a stream of nitrogen, and stored in glass. Whatman[2] 2V folded filter paper was washed with 100 ml chloroform-methanol (2:1) prior to use.

Skim milk was prepared by centrifugation of raw, whole herd milk in cellulose nitrate tubes at 1070 × *g* for 20 min at 4 C in a Spinco Model L Centrifuge. Following the spinning, the lower portion of each tube was punctured, and the skim milk was recovered, with care to exclude the cream layer. Sixty-milliliter samples were freeze-dried for analysis.

Isolation and Transmethylation of Cholesterol Esters

One hundred fifty milliliters of chloroform-methanol (2:1) were added to the freeze-dried skim milk (5.45 g), and the mixture was stirred magnetically for 3 h, then filtered. The powder was washed with an additional 100 ml of chloroform-methanol (2:1). The filtrates were combined, and the methanol was removed from the solvent mixture by extraction with two 90-ml portions of .9% aqueous potassium chloride solution and 90 ml of distilled water. The chloroform layer was dried with 65 g of sodium sulfate, then decanted, and evaporated to dryness under nitrogen at 37 C. The residue was taken up in 15 ml of methylene chloride and redried with 5 g of sodium sulfate. The solvent was transferred, in 2-ml increments, with a Pasteur pipette to a 45 × 15 mm screw capped vial and evaporated to dryness under a stream of nitrogen at 23 C. The residue was redissolved in 1 ml of methylene chloride and spotted on two .5 mm thick precoated silica gel G thin-layer chromatographic plates, which then were developed 15 cm with a hexane-benzene (3:2) solvent system. Following development, the plates were air dried, and the area corresponding to authentic cholesterol esters (cholesteryl butyrate and cholesteryl stearate) was scraped from the plates. The silica gel G was packed in a 10 × 1.8 cm glass column plugged with glass wool, and the cholesterol esters were eluted with 20 ml of

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[2] Reference to brand or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

methylene chloride. The eluate was dried with 5 g sodium sulfate and evaporated to dryness in a 60 × 17 mm screw capped vial. The cholesterol esters were transmethylated in 1 h in the sealed vial containing 5 ml of anhydrous methanol/1% sulphuric acid at 90 C. Recovery of the methyl esters and cholesterol was accomplished as in (2).

Mass Spectrometry

Mass spectra were obtained with the LKB 9000 Combination Gas Chromatograph-Mass Spectrometer at a constant accelerating voltage of 3.5 kv and electron energy of 70 ev. The mixture of methyl esters and cholesterol was chromatographed on a 1.22 m × .32 cm stainless steel column packed with 1% Se30 on 100-120 mesh Gas Chrom Q. The column temperature was programmed from 80 to 240 C at 4 C per min. The flash heater and molecular separator were maintained at 250 C, and helium served as the carrier gas.

Gas Chromatography Fatty Acid Quantitation

Gas-liquid chromatography was with the Perkin-Elmer Model 900 Gas Chromatograph equipped with a flame ionization detector. The methyl esters were separated isothermally at 120 C on a 1.83 m × .32 cm stainless steel column packed with 6% Silar 10-C on 60-80 mesh Gas Chrom Q. The flash heater and manifold were maintained at 230 C, and helium served as the carrier gas. Quantitative data were obtained by measuring peak areas and referring to detector response factors determined for known amounts of methyl esters.

RESULTS AND DISCUSSION

The isolated fraction was identified tentatively as cholesterol esters by thin-layer chromatography Rf and the formation of a characteristic red-pink color after the plates were sprayed with 50% aqueous sulphuric acid and heated slowly to 120 C. The fatty acids and cholesterol in the isolated fraction were identified conclusively by thin-layer chromatography Rf, gas chromatography retention times, and mass spectrometry following transmethylation.

Table 1 contains quantitative data on the fatty acid composition of the cholesterol esters of skim milk prepared from raw whole milk. The data, typical of numerous samples, reveal differences significant in the fatty acid composition of the cholesterol esters of skim milk compared to that in (1, 5) for the cholesterol esters of whole milk. Patton and coworkers (1, 5) reported octadecadienoic acid contents of 10 and 24 wt % in the cholesterol esters of whole milk while total unsaturated acids accounted for approximately 50 wt % of the fatty acid composition in each study. Our own limited studies on whole milk generally agree with their observations. In contrast, the cholesterol esters of skim milk are characterized by an octadecadienoic acid content greater than 70 wt % of the total fatty acid composition. In all, unsaturated fatty acids account for approximately 90 wt % of the cholesterol esters of skim milk. Only trace amounts of fatty acids with less than 14 or greater than 18 carbon atoms were observed.

The data reflect previous observations (3, 4) that unsaturated lipids have an apparent affinity for the aqueous phase of milk. Further studies will be necessary to determine whether this affinity is the result of unsaturated lipids being bound preferentially to the proteins of milk or because they are constituents of the membrane material of skim milk.

TABLE 1. Fatty acid composition of the cholesterol esters in skim milk.

Fatty acid	nMoles/60 ml of skim milk
$C_{14:0}$	2.0
$C_{14:1}$	.9
$C_{15:0}$	.3
$C_{15:1}$	tr*
$C_{16:0}$	7.7
$C_{16:1}$	10.1
$C_{17:0}$	tr*
$C_{18:0}$	1.1
$C_{18:1}$	4.9
$C_{18:2}$	83.2
$C_{18:3}$	5.0
Total	115.2

*Trace amounts.

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